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Unidentified chick growth factors in unsaturated fats

Donald Stanley Carver
Iowa State College

UNIDENTIFIED CHICK GROWTH FACTORS IN UNSATURATED FATS

by

Donald Stanley Carver

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Signature was redacted for privacy.

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Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

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INTRODUCTION

It has long been known that fat is an excellent source of energy for poultry. However, fat has never been considered an essential part of chick diets for maximum growth and feed utilization although this belief was founded on extremely limited research.

The trend in recent years has been to remove an increasing percentage of the fat from animal and vegetable protein ingredients such as soybean oil meal, cottonseed meal, and meat and bone scraps. At the present time many of the vegetable fats are being produced in quantities which exceed the demand. This raises the question as to the feasibility of removing such a large percentage of the fat when it may have exceptional value in the source material.

The following investigations were conducted to establish the requirement for fats, or possible factors present in fats, in the diet of the chick.

REVIEW OF LITERATURE

The Fat Requirement of the Chick

Extracting an all-mash starter with diethyl ether until the diet contained 0.1 per cent or less of ether extractables did not significantly retard chick growth up to 14 weeks of age according to Russell, Taylor and Polskin (1940). Davis and Upp (1941) reported that the extraction of an all-mash ration for 30 hours with isopropyl ether decreased the growth of chicks, but this slight difference disappeared by the time the pullets reached maturity.

Recently, Reiser (1950a) demonstrated that a fat-free, semi-purified ration produced poor livability and growth and that a level of 4 per cent cottonseed oil prevented these adverse effects. Reiser (1950b) also found that chicks could not synthesize linoleic or linolenic acids, but produced higher polyunsaturated acids from these.

Unidentified Growth Factors in Fats

Deuel, Meserve, Straub, Henrick and Sheer (1947) showed that rats receiving diets containing 5 to 50 per cent fat grew more rapidly, reached a greater final weight, had a greater capacity for exhausting work, attained sexual maturity earlier, were more fertile, and raised larger young than comparable rats fed diets devoid of fat. This was not the result of a fatty acid deficiency, or a deficiency of known fat-soluble vitamins, because diets with added methyl linoleate or ethyl laurate produced slow growth, poor fertility and late sexual maturity. The nature of the fat, whether cottonseed oil or oleomargarine, had little influence on the results. The most pronounced response was observed in males.

Scheer, Soule, Fields and Deuel (1947) confirmed the observation that a diet containing a natural fat caused earlier maturity and superior growth of weanling rats to one containing only methyl linoleate. Scheer, Codie and Deuel (1947), using the restricted isocaloric feeding technique, demonstrated a requirement of the rat for fat (cottonseed oil) over and above that of methyl linoleate.

The addition of 10 per cent cottonseed oil, oleomargarine, summer butter fat and olive oil to a fat-free diet was found by Lassen and Bacon (1949) to produce a significant growth stimulation in rats regardless of the simultaneous feeding of ethyl linoleate. All the fats produced similar growth responses.

Satisfactory growth of mice was obtained by Bosshardt, Paul, Barnes and Huff (1950) with a semipurified diet containing 0.5 per cent corn oil unless 2 per cent succinylsulfathiozole was included. The retardation of growth by succinylsulfathiazole was eliminated when fat or certain vegetable materials such as defatted cottonseed meal, rolled oats, or dried grass were added to the basal diet. They suggested that a factor essential for lipid synthesis is formed by the intestinal flora and that this factor is present in natural fats, cottonseed meal, oats and grass.

Peterson (1950), working with chicks, found that the growth depressing effect of 20 per cent alfalfa meal was completely overcome by the inclusion of both 0.2 per cent cholesterol and 4 per cent cottonseed oil.

Kaunitz and Slanetz (1950) fed rats a vitamin A-free diet and were able to protect them from vitamin A deficiency symptoms by the addition to the diet of a lard distillate fraction. This fraction was shown by analysis to be very low in tocopherols and vitamin A. It was concluded that the distillate fraction of lard contained a factor with vitamin A-like activity but which was different from the known forms of vitamin A.

Suckling rats made more rapid gains when their mothers were fed corn oil than when fed a fat-free, purified diet according to Loosli, Lingenfelter, Thomas and Maynard (1944). No growth response occurred in suckling rats when their lactating mothers were given completely hydrogenated coconut oil or 125 milligrams of ethyl linoleate per day.

Keane, Cohn and Johnson (1951) have shown that female rats fed a diet containing glyceryl trilaurate plus methyl linoleate as fat sources were unable to rear their young to weaning age. However, when this diet was supplemented with 2.5 per cent of wheat germ oil or corn oil, the young were raised with success. Female rats, which had grown to maturity on a fat-free diet and bred, gave birth to young which were born dead or died soon after birth according to Kummerow, Pan and Hickman (1952). When this diet was supplemented with 5 per cent hydrogenated fat (Crisco) the animals gave birth to living young which did not survive more than 72 hours, while dams fed 5 per cent corn oil weaned 85 per cent of their young.

Essential Fatty Acids

Burr and Burr (1929) were the first to report a requirement of polyunsaturated fatty acids by the rat for prevention of scaly skin, caudal necrosis, retardation of growth, kidney lesions, reproductive failure and high mortality. Of these effects, growth response and skin cure were the best measures of effectiveness of oils and fatty acids according to Burr and Barnes (1943). Burr, Brown, Kass and Lundberg (1940) reported that 20 mg. of corn oil, linseed oil or cod liver oil gave appreciable growth response, but only corn oil produced a complete skin cure. Linoleic acid in the corn oil was responsible for the correction of skin abnormalities.

Engel (1942) demonstrated that polyunsaturated fatty acids were required to prevent fatty livers in rats. Quackenbush, Steenbock, Kummerow and Platz (1942) found that linoleic acid was more indispensable than pyridoxine and pantothenic acid in preventing rat dermatitis.

Burr and Barnes (1943) state that only linoleic acid is actually essential in the rat's diet since linolenic and arachidonic acids can be synthesized from linoleic. They found that 50 to 100 milligrams of linoleic acid per day would satisfy the rat's requirement. The relative effectiveness of arachidonate versus linoleate was found to be a ratio of 3.5:1 by Greenberg, Calbert, Deuel and Brown (1951).

Mice developed a dandruff-like dermatitis and later the tissues of the extremities became necrotic when fed fat-free rations. White, Foy and Cerecedo (1943) could prevent or cure this condition with the addition of 0.10 per cent lard to the ration. Decker, Fillerup and Mead (1950) stated that corn oil prevented fatty livers and dermatitis of mature mice receiving a fat-free diet. The need for fat was more critical in the case of the male animal.

It required 42 days for weanling pigs, on a ration containing 0.06 per cent fat, to develop deficiency symptoms according to Witz and Beeson (1951). Typical fat-deficiency necrotic areas were noted on the shoulders and necks of the pigs receiving the fat-free ration. Growth was retarded but there was no difference in the degree of fatness of the pigs receiving the fat-free or the fat supplemented diet. Corn oil cured the deficiency symptoms.

Hansen and Burr (1946) stated that no specific clinical syndrome developed in human infants fed a fat-free ration during a period of one to two years.

Hansen and Wiese (1943) reported that weaned puppies developed a skin syndrome indicative of an essential fatty acid deficiency after receiving a fat-free diet for three months. The inclusion of lard in the diet prevented the dermatitis of the skin, but did not improve the growth of the puppies.

EXPERIMENTAL PROCEDURE

Stock Used

Day-old White Leghorn or New Hampshire chicks from the Iowa State College Poultry Farm flock were used in the experiments. In most of the experiments the chicks were vent-sexed by commercial chick sexors. Straight-run chicks were used in Experiments I-V, VI, VII; only females in Experiments VIII, IX, X, XII, XIII; only males in Experiments XI, XVII; and half of each sex were used in Experiments XV, XVI, XVIII, XIX. Except when otherwise indicated, two lots of ten chicks each were fed each experimental diet.

Methods of Feeding and Management

The experiments were conducted in a battery brooder equipped with wire floors and thermostatically controlled electric heating elements of the back-warmer type. The temperature under the hovers was adjusted to the comfort of the chicks from an initial temperature of 110° F. The hovers were stationary at a height of four inches throughout the four week period. The battery room temperature was maintained at approximately 75° F. except during the summer months when the temperature was occasionally as high as 90° F.

Diets were fed *ad libitum* throughout the experiments and water was available at all times. At the start of each experiment sufficient vitamin-premix and feed for the test period were mixed for each lot and stored in metal cans with tight-fitting covers. Vitamins were supplied as a vitamin premix consisting of 50 per cent dextrose and 50 per cent cellulose. Diets in Experiment XIX were refrigerated at 44° F. while in all other experiments feed was kept in the battery room. Supplements to the basal diet were stored at 44° F. previous to incorporation in experimental diets.

Basal Diets and a Description of Supplements

The basal diets are shown in Table 1. Supplements to the basal diets were added at the expense of dextrose. The industrial soybean protein basal diet was used in the experiments unless otherwise indicated. The following is a list of the experiments indicating the mineral mix and level of industrial soybean protein incorporated in the basal diet:

Mineral mix A 5.0 per cent : VI, VII, VIII, X
Mineral mix A 7.0 per cent : IX, XI
Mineral mix B 5.5 per cent : XII through XIX

Industrial soybean protein 24 per cent : VI through XI
Industrial soybean protein 25 per cent : XII through XIX

The 25 per cent soybean protein basal diet contained 21.44 per cent protein by analysis (Nitrogen x 6.25), while the 24 per cent soybean protein basal and the vitamin test-casein

Table 1

Composition of Basal Diets

Ingredients	Industrial Soybean Protein	Vitamin-test Casein
Drackett industrial protein 220	24.00 (25.00)[2]	-----
Vitamin-test casein	-----	24.20
Dextrose-tech.	65.21 (63.71)	63.87
Cellulose	5.00	5.00
Mineral mix[1]	5.00 (5.50)	5.00
Choline chloride	0.15	0.15
DL-methionine	0.64	-----
L-arginine	-----	0.38
L-cystine	-----	0.40
Glycine	-----	1.00
Fat-content (by analysis)	0.04 (0.04)	0.05

The following vitamins[3] were added per pound:

Vitamin A palmitate, I.U.	6,000	Biotin, mg.	0.15
Vitamin D_3, I.C.U.	566	Vitamin B_{12}, mcg.	30
Thiamin hydrochloride, mg.	2.7	Menadione, mg.	5.4
Riboflavin, mg.	4.8	Alpha tocopherol acetate, mg.	10
Calcium pantothenate, mg.	15	Folic acid, mg.	1.05
Niacin, mg.	30	Para-aminobenzoic acid, mg.	68.1
Pyridoxine hydrochloride, mg.	4.8		
I-inositol, mg.	90		

[1]Mineral mix A (at the 5 per cent level) or B (at the 5.5 per cent level) provided the following per pound of diet:

	A	B
Ca %	0.9	1.0
P %	0.5	0.6
NaCl %	0.6	0.6
K %	0.3	0.25
Mg, mg.	431	239
Zn, mg.	64	76
Co, mg.	0.4	2
Fe, mg.	82	101
Mn, mg.	32	38
I, mg.	5	13
Cu, mg.	2	3

[2]Figures in parenthesis represent level of the ingredient in the 25% industrial soybean protein basal.

[3]Crystalline vitamins except folic acid and vitamin D

basal contained a calculated 20.58 and 21.61 per cent protein, respectively. The fat content of the basal diets is given in Table 1.

A description of the concentrates and other special supplements used in this series of experiments follows:

1. Sixty Per Cent Linoleic Acid Concentrate[1] is a 60 per cent concentrate prepared from linseed oil by fractional distillation with the remaining 40 per cent being mostly oleic acid.

2. Oleic Acid Concentrate[1] is obtained by the hydrolysis of tallow. It has an iodine value of 85-95 and contains small amounts of other unsaturated fatty acids.

3. Liver L[1] is a solubilized dry concentrate of bovine liver containing the alcohol insoluble fraction. Methanol was employed.

4. Biopar C[2] is the hot water soluble and 70 per cent alcohol insoluble fraction of pork liver.

5. Pork Liver Residue[2] is the hot water insoluble fraction of the liver which contains 20.2 per cent of alcohol-ether (3:1) soluble material. Seventeen per cent of its total fatty acids are linoleic and 18.8 per cent are arachidonic acid.

6. Oleomargarine[3] (Experiment XIX) had the following composition (%): soybean oil 80.00, salt 3.10, lecithin 0.20, skim milk solids 1.40, mono- and diglycerides 0.20, skim milk and moisture 15.10.

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were provided through the courtesy of Merck and Co., Inc. Folic acid was provided by Lederle Labs., Inc. and vitamin D_3 was made available by the E. I. du Pont de Nemours and Co.

[1]Nutritional Biochemical Corporation, Cincinnati, Ohio.

[2]Courtesy of Armour and Company, Chicago, Illinois.

[3]"Nu-Maid", The Miami Margarine Co., Cincinnati, Ohio.

Table 2 contains the unsaturated fatty acid composition of the fats and oils used in these experiments.

Records and Experimental Design

Both chicks and treatments were assigned at random to the experimental pens. Two experimental designs were employed. Experiments XV, XVII, XVIII and XIX involved a randomized complete block design, while all other experiments were of the completely randomized type. Chicks and feed were weighed at weekly intervals and mortality was recorded daily. In all cases where a pen contained half males and half females, the average chick weight was obtained by totaling the means of the two sexes and dividing by two. The analysis of variance of chick weight and feed efficiency data of all experiments which had replicated treatments was made according to the method reported by Snedecor (1946).

Chemical Tests and Special Procedures

The procedure for the saponification and separation of saponifiable and nonsaponifiable fractions of the cottonseed oil in Experiments XVI and XVII was described by Hilditch (1947). Kerr (1918) described the Kreis test for rancidity of oils in which the fat is shaken in a dilute ethereal solution of phloroglucinol in the presence of hydrochloric

Table 2

The Unsaturated Fatty Acids of the Vegetable Oils and Other Fats

Oil or Fat	Per Cent of Total Fatty Acids			
	Oleic	Linoleic	Linolenic	Arachidonic
Cottonseed oil (1)	22.9	47.8	-	-
Corn oil (2)	30.1	56.3	-	
Wheat germ oil (1)	12.0	57.0	9	-
Soybean oil (1)	27.0	56.0	4	-
Linseed oil (1)	23.0	15.0	52	
Beef tallow (1)	48.0	3.0	-	traces
Lard (3)	47.7	12.5	0.57	0.39
Butter (1)	32.4	4.0	-	0.4
Liver residue (pork) (4)		17.0	-	18.8

(1) Hilditch (1947)

(2) Baur and Brown (1945)

(3) Stillman, Ferguson, Daubert, Milner, O'Connor, and Beadle (1948)

(4) Allen (1952)

acid, the intensity of the pink color produced being a measure of the amount of aldehydes present. Iodine value determinations were conducted according to the Rosenmund-Kuhnhenn method described by Yasuda (1931) and this value is a measure of the mean unsaturation of a fat. Crude fat and protein in the feed were determined according to the methods of the Association of Official Agricultural Chemists (1945).

In Experiment XVI the procedure for the carcass composition study was as follows: Chicks were bled by decapitation and then dipped in 134° F. water for several minutes before removing the feathers. The portion of the legs from the hock joint to the toes was removed. The alimentary canal was extirpated except for the gizzard. The remainder was passed through a Hobart meat grinder. This material was dried at 110° C. for 48 hours. The dried residue was pulverized and mixed in a Waring Blendor for several minutes and weighed. This was followed by Soxhlet extraction with a 3:1 alcohol-ether solution for 48 hours and subsequently the small amount of alcohol and ether remaining in the carcass material was evaporated by heating for 24 hours at 100° C. The per cent fat of the moisture-free carcass was calculated from the difference in weight of the carcass material before and after extraction. In Experiment XVII the same procedure was followed except the chicks were skinned and each carcass was dried initially at 135° C. Kjeldahl determinations were conducted on the moisture-free, fat-free, carcass using the method of the Association of Official Agricultural Chemists (1945).

EXPERIMENTAL RESULTS

Experiments I-V

Objective

Five preliminary experiments were conducted to develop a semi-purified, "fat-free" basal diet for chicks which would be satisfactory for studying the nutritive value of fats and fatty acids.

Methods

Small groups of New Hampshire chicks were fed the experimental diets to three or four weeks of age. Initially, a 50 per cent protein soybean meal which was extracted with trichloroethylene for a 24-hour period was used as a source of protein. Extraction for an eight-hour period with hot ethyl alcohol or extraction with hot ethyl alcohol for a 24-hour period and subsequently extraction with trichloroethylene for a 48-hour period were employed in two separate experiments. Vitamin-test casein also was used as a source of low-fat protein.

Results

Tables showing the results of the preliminary experiments are not included because the results were negative and followed a pattern which can be adequately summarized in a few sentences. These experiments demonstrated that vitamin-test casein, and especially extracted soybean oil meal, were not satisfactory as low-fat sources of protein.

A 60 per cent concentrate of linoleic acid retarded growth and caused the following symptoms characteristic of a vitamin E deficiency in chicks when added to the basal diet containing either of the above sources of protein: anorexia, loss of equilibrium, retracted heads, leg tremors, cerebellum hemorrhages and death. The symptoms appeared when the chicks were about 15 days old. Nearly 100 per cent of the chicks died before reaching 30 days of age. As the level of the linoleic acid concentrate in the diet was raised from 0.5 to 6.6 per cent, the toxicity was increased and the appearance of deficiency symptoms was accelerated.

Experiment VI

Objective

Experiment VI was conducted to determine whether "fat-free" diets containing either industrial soybean protein or vitamin-test casein would support satisfactory growth when supplemented with refined corn oil.

Methods

Nine day-old straight-run New Hampshire chicks were started in each pen.

Results

Figure 1 shows the results of supplementing the basal diets with refined corn oil. The addition of 4 per cent corn oil to either diet increased body weight at four weeks of age by approximately 30 per cent. The industrial soybean protein basal with or without the corn oil was superior to the vitamin-test casein basal diet.

The difference in weight between the industrial protein fed chicks and chicks fed the casein was magnified as age increased. The difference between the two groups was 20.0, 23.9, 27.5 and 29.6 per cent at 1, 2, 3 and 4 weeks of age, respectively.

Mortality was low in all experimental groups.

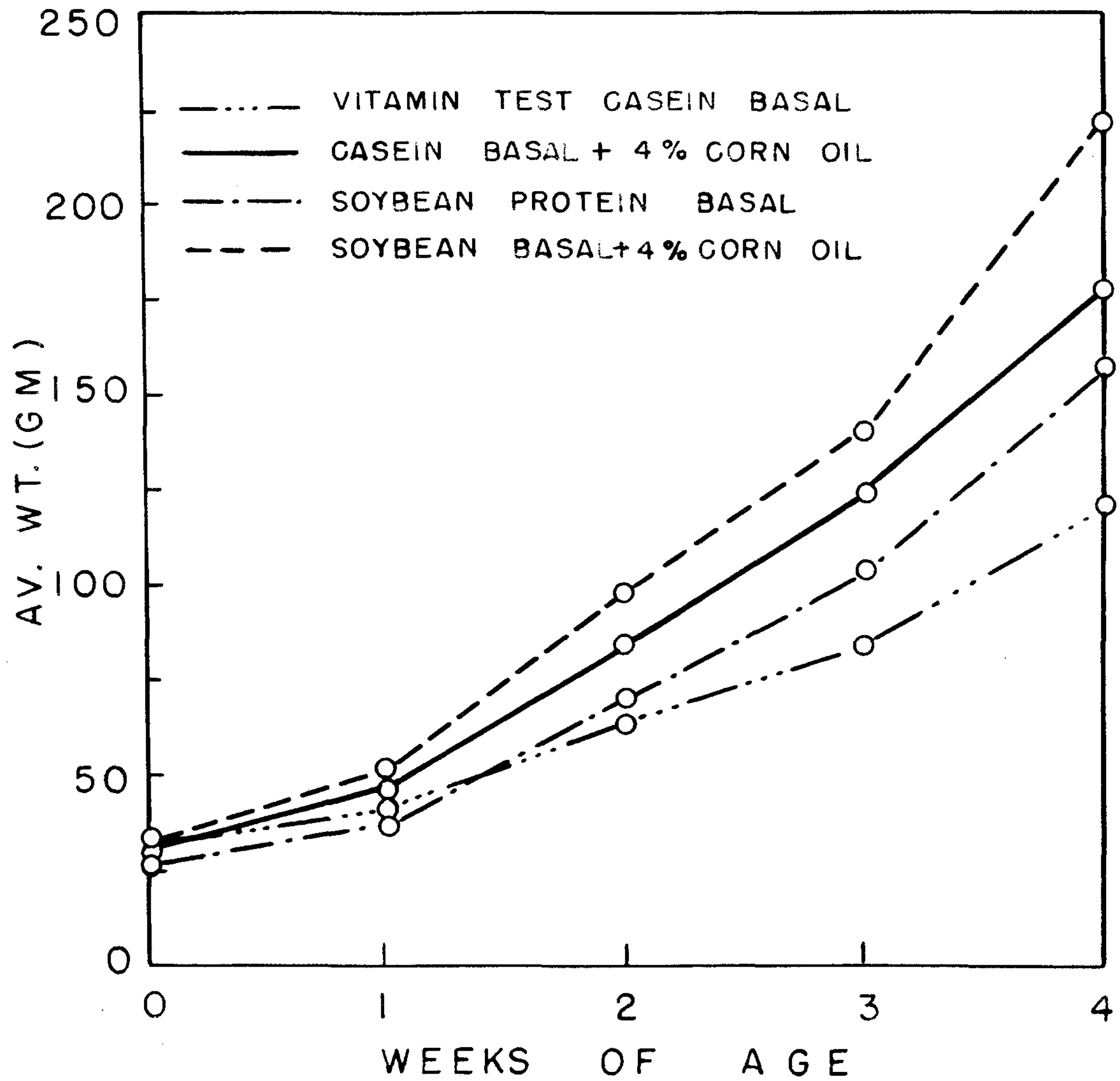

Figure 1. INDUSTRIAL SOYBEAN PROTEIN SUPERIOR TO VITAMIN - TEST CASEIN IN BASAL FOR CHICKS.

Experiment VII

Objective

Experiment VI indicated that refined corn oil provided growth-promoting substances for the chick when present in the diet at the 4 per cent level. The objective of Experiment VII was to confirm the response to refined corn oil and to test crude corn oil and soybean oil as sources of the growth-stimulating factors.

Methods

Twelve, day-old, straight-run New Hampshire chicks were started in each pen.

Results

As shown in Table 3, improved growth and feed efficiency were obtained using 4 per cent refined corn oil, crude corn oil or soybean oil. The two corn oils were slightly superior to the soybean oil in improving chick growth. The feed efficiency for the corn oil and the soybean oil groups was essentially the same.

Mortality was low in all groups and all chicks in the group receiving the "fat-free" diet survived the four-week period.

Table 3

Unidentified Factors in Vegetable Oils Increase Chick Growth

Modification of diet (%)	Av. wt. in gm. 4 wk.	F.E.[1]
Basal	136	2.85
4 refined corn oil	205	2.23
4 crude corn oil	201	2.25
4 soybean oil	184	2.21

[1]Grams of feed per gram of gain. Experiment VII

Experiment VIII

Objective

Previous experiments (I-V) had demonstrated that the addition of a 60 per cent concentrate of linoleic acid to either the vitamin-test casein basal or the specially extracted soybean oil meal basal produced detrimental effects. Since linoleic acid is the principle fatty acid of corn oil and soybean oil, Experiment VIII was initiated to determine whether the linoleic acid concentrate would increase growth equivalent to crude corn oil or be toxic when added to the industrial soybean protein basal. A further objective of this experiment was to determine whether either of two antioxidants would counteract the deficiency condition previously produced by the linoleic acid concentrate. Since oleic acid is also present in varying concentrations in vegetable oils, it was desirable to ascertain whether this fatty acid was responsible for the increase in growth caused by the vegetable oils in Experiments VI and VII. Wheat germ oil was also tested for growth factor activity.

Methods

Ten female White Leghorn chicks were started in each of ten pens. Half of the chicks in each pen were sacrificed at 32 days of age and their livers were removed and weighed.

Results

The 60 per cent concentrate of linoleic acid was not toxic at the levels fed in the presence or absence of wheat germ oil or alpha tocopherol. One and one-half per cent of the linoleic acid concentrate was more effective for increasing growth than the other levels employed as shown in Table 4. The chicks receiving either the 1.5 per cent linoleic acid concentrate or 4 per cent crude corn oil had the same average body weight at 31 days of age.

The following oils produced approximately 28 per cent heavier chicks than the basal at 31 days of age: 1.5 per cent oleic acid concentrate; 1.5 per cent wheat germ oil; a

Table 4

Fatty Acid Concentrates and Vegetable Oils Supplement "Fat-free" Diet

Modification of diet (%)	Av. wt. at 31 days (gm)
Basal	167
4.000 crude corn oil	216
0.025 wheat germ oil	198
0.250 wheat germ oil	187
1.500 wheat germ oil	231
1.500 oleic acid concentrate	235
0.025 crude linoleic acid conc.	200
0.250 crude linoleic acid conc. + 0.250 wheat germ oil	230
1.500 crude linoleic acid conc.	216
0.500 crude linoleic acid conc. + 0.025 alpha tocopherol	201

Experiment VIII

combination of 0.25 per cent wheat germ oil and 0.25 per cent linoleic acid concentrate.

Mortality was low in all lots. Liver weights were not significantly affected by the fat supplements.

Experiment IX

Objective

In Experiment VIII 1.5 per cent of the oleic concentrate was found to be a potent source of unidentified growth factors for the chick. The objectives of Experiment IX were to determine: (1) the response to several levels of the oleic acid concentrate; (2) whether the oleic acid concentrate and the linoleic acid concentrate contained separate growth factors; (3) whether soybean oil and refined corn oil contained separate growth factors.

Methods

Two lots of ten female White Leghorn chicks were started on each experimental diet.

Results

The results of this experiment are presented in Table 5.

Table 5

Oleic Acid Concentrate Superior as a Supplement to a "Fat-free" Diet

Modification of diet (%)	Av. wt. at 3 wk. (gm)[1]
Basal	109
0.25 oleic acid concentrate	120
1.00 oleic acid concentrate	134
2.00 oleic acid concentrate	132
0.25 oleic acid concentrate + 0.25 crude linoleic acid conc.	123
0.25 crude linoleic acid conc.	105
2.00 soybean oil + 2.00 refined corn oil	121
2.00 refined corn oil	120
2.00 soybean oil	121
2.00 cod liver oil	118

Experiment IX

[1]Significant mean difference at the 5% level of probability is 22 gm. when compared with the basal.

An increase in the level of oleic acid concentrate from 1 to 2 per cent in the diet did not increase chick weight appreciably at three weeks of age. However, 0.25 per cent oleic acid concentrate was not adequate and growth was only slightly improved by a similar level of linoleic acid concentrate.

Soybean oil and refined corn oil did not appear to contain separate growth factors since a combination of 2 per cent of each oil gave no additional growth over either oil fed individually.

Mortality was low in all groups except those receiving 2 per cent cod liver oil in which a 35 per cent loss occurred. A vitamin D deficiency was quite evident by four weeks in all lots except the two receiving cod liver oil. The cause of the deficiency is not known, but a new supply of crystalline vitamin D_3 was used in subsequent experiments which resulted in no further deficiency symptoms. Since the symptoms of this deficiency were not noticeable at three weeks, it is doubtful that this deficiency influenced the outcome of the results reported in Table 5.

Experiment X

Objective

Since the crude 60 per cent linoleic acid concentrate had proved toxic as a supplement to the vitamin-test casein basal and erratic as a growth-promoter for the industrial casein basal, Experiment X was conducted to determine whether the toxic portion predominated in the liquid oil or the solid particles which settled to the bottom of the containers. Another objective of this experiment was to study the effect of "fat-free" diets on liver weight.

Methods

The linoleic acid concentrate was filtered through several layers of cheesecloth to remove all the solid particles. Four grams of settlings (solid particles) were added to each of the diets that are listed in Table 6 as containing settlings. Twelve, day-old female White Leghorn chicks were started on each of the experimental diets. At four weeks of age all surviving chicks were slaughtered and their livers removed. The livers were heated in a drying oven for 24 hours at 95 degrees C. before being weighed.

Table 6

A Difference in Response to the 60 Per Cent Linoleic Acid Concentrate with the Casein and Soybean Protein Basal Diets

Modification of basal	Chick wt.[1] 24 days old	% mortality 26 days	% liver[2] of total body wt.
Industrial soybean protein basal	164	0.0	1.94
Industrial soybean protein basal + 1.50% Linoleic acid conc. (free of settlings)	192	8.3	1.30
Industrial soybean protein basal + 1.50% Linoleic acid conc. (containing settlings)	191	0.0	1.20
Vitamin-test casein basal	139	0.0	2.10
Vitamin-test casein basal + 1.50% linoleic acid conc. (free of settlings)	100	33.3	1.30
Vitamin-test casein basal + 1.50% linoleic acid conc. (containing settlings)	128	33.3	1.50

Experiment X

[1] Twelve chicks started on each treatment.

[2] With moisture removed from livers.

Results

The linoleic acid concentrate was toxic only when added to the vitamin-test casein basal, as shown in Table 6. There was an average mortality of 4.1 per cent for the two groups receiving the industrial soybean protein and linoleic acid concentrate while an average of 33.3 per cent of the chicks died when the concentrate was added to the vitamin-test casein basal. The toxicity of the linoleic acid concentrate was the same in the presence or absence of the settlings. No mortality occurred in either of the "fat-free" basal groups. The addition of linoleic acid concentrate to the industrial soybean protein basal increased growth by approximately 15 per cent. The two "fat-free" basal diets did not cause enlarged livers.

Experiment XI

Objective

Since linseed oil contains relatively large amounts of two of the "essential" fatty acids for the rat, linoleic and linolenic acids, an objective of Experiment XI was to determine whether linseed oil contained chick growth-promoting factors. A further objective was to determine whether procaine penicillin would stimulate growth of chicks when present in a "fat-free" diet.

Methods

Twenty White Leghorn male chicks were started on each experimental diet. At two and three weeks of age each chick was given orally 100 International Chick Units of crystalline vitamin D_3 in ether solution to avoid a possible vitamin D deficiency.

Results

Linseed oil retarded growth of chicks as shown in Table 7. Seventy-one per cent of the chicks receiving 2 per cent

Table 7

Linseed Oil Toxicity Eliminated by Refined Corn Oil

Modification of basal (%)	Average gain in grams[1] 2-3 wk.	3-4 wk.	% mortality at 4 wks.
Basal	33	45	0
10 p.p.m. procaine penicillin	44	60	10
2 linseed oil	26	-3	71
2 refined corn oil	43	44	5
2 linseed oil + 2 refined corn oil	36	52	6

Experiment XI

[1]Twenty chicks started in each group.

linseed oil in the diet died by the fourth week in comparison with 100 per cent livability in the basal group. The symptoms of these chicks were the same as previously observed when the linoleic acid concentrate was added to the vitamin-test casein basal -- those characteristic of a vitamin E deficiency. The addition of 2 per cent refined corn oil to the diet containing linseed oil eliminated most of the reduction in growth and mortality.

The two-to-three week average weight gain figures in Table 7 present a more accurate picture of the response to the refined corn oil addition, because the chicks in this one group developed inflammation of the eyes during the three-to-four week period due to a bacterial infection. The infection reduced the growth rate of these chicks. The figures for two-to-three week average gains indicated that penicillin increases growth of chicks in the absence of fat in the diet, and that the improvement in growth is the same with either penicillin or corn oil.

Experiment XII

This experiment was initiated to (1) establish the minimum level of the oleic acid concentrate which would give a maximum response; (2) to determine whether Liver L contained the factors present in the oleic acid concentrate;

(3) and to study several combinations of wheat germ oil and linoleic acid concentrates.

Methods

Two pens of ten female White Leghorn chicks each were started on each diet.

Results

Experimental results are shown in Figure 2 and Table 8. Growth response to the oleic acid concentrate was erratic within replicate lots at the lower levels of feeding. Very little response and even negative results were obtained from the chicks in the pens on the sunny side of the battery brooder which was probably due to slight rancidity of the oil in the feed. According to Kerr (1918) heat and light catalyze rancidity. The 1.25 per cent level of oleic acid concentrate gave a substantial increase in growth over the basal. Liver L increased growth to a similar extent when added to the basal. Liver L and the oleic acid concentrate appeared to contain separate and distinct growth factors, since only a combination of the two supplements produced maximum growth. As indicated in Figure 2, a combination of 2.0 per cent Liver L and 1.25 per cent oleic acid concentrate produced significantly greater growth than the basal.

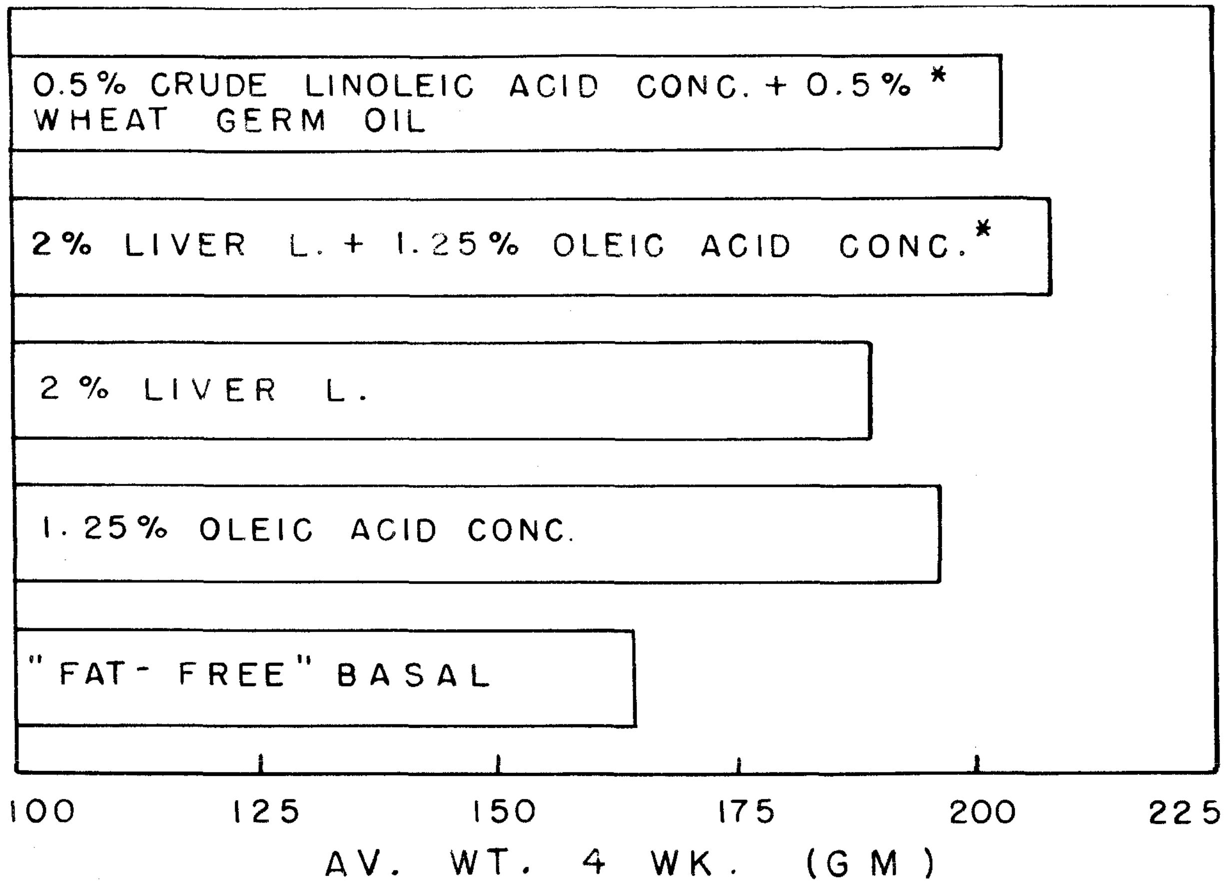

Figure 2

GROWTH-PROMOTING ACTION OF LIVER L. AND OLEIC ACID CONCENTRATE IS ADDITIVE.

Table 8

The Inconsistent Response to Oleic Acid Concentrate at Low Levels in the Diet

Modification of basal (%)	Av. wt. 4 weeks Replicate I	II	Av.[1]
Basal	168	160	164
0.50 oleic acid conc.	179	144	161
0.75 oleic acid conc.	172	156	164
1.00 oleic acid conc.	193	152	172
1.25 oleic acid conc.	211	183	197
2.00 Liver L	174	204	189
2.00 Liver L + 1.0 oleic acid conc.	219	196	208
0.25 (60%) linoleic acid conc. + 0.25 wheat germ oil	180	174	177
0.50 (60%) linoleic acid conc. + 0.50 wheat germ oil	218	188	203
1.00 (60%) linoleic acid conc. + 1.00 wheat germ oil	113	188	150

Experiment XII

[1]Twenty chicks per treatment.
Significant mean difference at the 5% level, as compared to the basal, is 41 gm.

The combination of 0.5 per cent wheat germ oil and 0.5 per cent linoleic acid concentrate was equal to the combination of Liver L and oleic acid concentrate in supplying unidentified growth factors. The negative results obtained with 1 per cent each of the linoleic acid concentrate and wheat germ oil in Replicate I are difficult to explain, but were probably due to an error in the mixing of the diet. The chicks in Replicate II of this treatment weighed 20 grams more than the chicks receiving the basal diet.

Experiment XIII

Objective

The 60 per cent linoleic acid concentrate and the oleic acid concentrate demonstrated growth-promoting properties in former experiments. Experiment XIII was done to ascertain whether these growth responses were due to high levels of linoleic and oleic acid in these concentrates or to the unidentified factor content. Stearic acid was also present in most of the supplements that were used previously. Therefore, a second objective of this experiment was to determine whether stearic acid was responsible for the growth-stimulating action of certain fats.

Methods

Two pens of ten female White Leghorn chicks each were assigned to each diet. The number of chicks receiving each oral supplement is indicated in Table 9. Methyl linoleate and methyl oleate were pure fatty acids. All oral supplements were fed by syringe twice weekly.

Table 9

Methyl Linoleate Increases Chick Growth

Oral supplement (Approx. %)[1]	No. chicks	Wt. 4 wk. (gm)
None		203
0.2 methyl oleate	1	188
1.0 methyl oleate	2	208
0.2 methyl linoleate	1	216
1.0 methyl linoleate	2	234

Experiment XIII

[1]Percentage is based on an assumed consumption of 454 grams of feed per chick by four weeks.

Results

The results shown in Table 9 demonstrate that the two chicks receiving 1 per cent of the pure methyl linoleate were 14 per cent heavier at four weeks than chicks receiving only the basal. The methyl oleate appeared to have no effect on growth, but not enough chicks were fed the pure fatty acid esters to reach definite conclusions.

Results shown in Table 10 show that stearic acid, a saturated 18-carbon fatty acid, does not increase chick growth, when fed at the level of 1 or 2 per cent. One and 2 per cent oleic acid concentrate improved the growth of chicks over the basal by 18.2 and 16.1 per cent, respectively.

Table 10

Stearic Acid Does Not Promote Chick Growth

Modification of diet (%)	Av. wt. at 4 wk. (gm)[1] Replicate I	II	Ave.
Basal	202	204	203
1.0 stearic acid	197	217	207
2.0 stearic acid	214	176	195
1.0 crude linoleic acid conc.[2]	217	209	214
1.0 oleic acid conc.	266	230	248
2.0 oleic acid conc.	248	235	242

Experiment XIII

[1]Significant mean difference at the 5 per cent level equals 42 gm. when compared with basal.

[2]Sucrose was used as a source of carbohydrate in place of dextrose in these two pens.

Experiment XIV

Objective

Experiments VIII, IX, XII and XIII had shown that the oleic acid concentrate was a potent source of unidentified growth factors for chicks. Since yellow corn is the principal source of fat in a corn-soybean type diet, Experiment XIV was conducted to determine whether yellow corn contained the unidentified factors present in the oleic acid concentrate.

Methods

Two pens of ten female White Leghorn chicks each were randomly distributed to each experimental diet. The Liver L was dispersed in water before being mixed in the diet.

Results

Experiment XIV was conducted during the warmest part of the summer (July 8 to August 5). The possible effects of weather on the outcome of this experiment will be discussed later. As indicated in Table 11, oleic acid concentrate decreased growth when it was added at the 1 per cent level to the basal. Corn did not improve feed efficiency but produced a slight growth response. The combination of the oleic acid concentrate and corn produced chicks which weighed

Table 11

Growth-Retarding Effect of the Oleic Acid Concentrate Under Warm Environmental Conditions

Modification of basal	Feed efficiency[2] 0-3 wk. Replicates			Chick wt.[1] 3 wk. Replicate		
	I	II	Av.	I	II	Av.[3]
Basal	1.88	1.93	1.90	163	176	169
1.0% oleic acid conc.	1.89	2.25	2.07	148	148	148
25.0% ground yellow corn	1.90	1.91	1.90	173	187	180
1.0% oleic acid conc. + 25.0% ground yellow corn	1.89	1.71	1.80	188	194	191
1.0% oleic acid conc. + 1.0% Liver L	2.20	2.31	2.26	172	165	169

Experiment XIV

[1]Significant mean difference at the 5% level equals 25 gm. when compared with the basal.

[2]Grams feed per gram of gain.

[3]Twenty chicks.

11.5 per cent more than the basal at three weeks of age. The combination of Liver L and oleic acid concentrate was not growth-promoting under these warm environmental conditions. Livability was 100 per cent in all lots.

Experiment XV

Objective

The objectives of this experiment were to determine: (1) whether unsaturated fats actually contained growth factors necessary for tissue metabolism or were only affecting intestinal absorption of nutrients such as the fat-soluble vitamins (based on the assumption that the separate administration of the fat from the feed once a day, four times weekly would eliminate the possible effects of fats on absorption); (2) whether the effects of wheat germ oil and oleic acid concentrate on growth were additive.

Methods

Two lots of five male and five female White Leghorn chicks were started on each diet. Each orally administered fat was placed in the chick's esophagus four times weekly with a syringe.

Results

Supplementing the basal with 2 per cent wheat germ oil in the feed, wheat germ oil given orally, or 1 per cent oleic acid concentrate in the feed, produced approximately the same increases in body weight over the basal -- about 10 per cent as shown in Table 12. When the oleic acid concentrate was given as an oral supplement, it lost its ability to improve growth. A combination of one per cent wheat germ oil plus 1 per cent oleic acid concentrate mixed in the feed was almost twice as effective as either supplement by itself.

Chicks receiving the fat-soluble vitamin mix with or without the oleic acid concentrate weighed almost the same as those on the basal diet. It is apparent that the level of vitamins A, D, E and K in the basal diet were adequate. The 60 per cent linoleic acid concentrate was growth-depressing when fed either orally or in the mixed feed, but more so in the latter case. When it became apparent that the linoleic acid concentrate was depressing growth, a Kreis rancidity test, as modified by Kerr (1918), was carried out on a sample of the oil. The test indicated the linoleic acid concentrate was in a late stage of incipient rancidity.

No mortality occurred in the groups receiving the "fat-free" basal diet.

Table 12

Growth Response to Oils Fed Orally or as Part of Mixed Feed

Modification of basal	Per cent increase[1]	Av. wt. 2 wk.[2]
Basal	0.0	90
2.0% wheat germ oil	11.2	102
1.0% oleic acid concentrate	10.0	100
1.0% wheat germ oil + 1.0% oleic acid concentrate	18.2	110
2.60 cc. wheat germ oil/chick/week	10.9	101
1.08 cc. oleic acid conc./chick/week	-4.4	86
Fat-soluble vitamin mix (0.6 cc./chick/week)[3]	0.0	90
Fat-soluble vitamin mix (0.6 cc./chick/week) + 1.12 cc. of oleic acid conc.	1.1	91
2.0% (60%) linoleic acid conc.	-13.1	78
2.4 cc. (60%) linoleic acid conc. /chick/week	-7.8	83

[1]Per cent increase in body weight over the basal at two weeks of age.

[2]Significant mean difference at 5% level when compared with the basal equals 16 gm.

[3]0.6 cc. of fat-soluble vitamin mix contained 924 I.U. of vitamin A palmitate, 62 I.C.U. of vitamin D_3, 0.25 mg. each of menadione and alpha tocopherol.

Experiment XVI

Objective

Experiment XVI was conducted to determine (1) whether pure methyl linoleate and methyl linolenate were growth factors for the chick; (2) whether pork liver residue, which is high in arachidonic acid, would promote chick growth; (3) whether both the saponifiable and nonsaponifiable fractions of cottonseed oil had growth-promoting properties; (4) whether replacing most of the dextrose in the basal diet with ground yellow corn would increase chick growth (indicating unidentified factors in corn which might be fat-soluble); and (5) whether the addition of various fats to the basal diet would cause an increase in carcass protein or carcass fat.

Methods

Two lots containing five male and five female White Leghorn chicks were started on each experimental diet, except for those receiving the pure esters of the fatty acids whose number of chicks per lot is indicated in Table 13. Oral supplements of the methyl esters of the fatty acids were administered daily by syringe.

Table 13

Pure Methyl Linolenate Stimulates Chick Growth

Modification of basal	No. of chicks at start	Av. wt. (gm) 4 wk.[1]
Basal	16	133
Methyl linoleate[2]	10	107
Methyl linoleate[3]	6	117
Methyl linolenate[2]	10	164
Methyl linolenate[3]	6	150

Experiment XVI

[1]Average weight males plus average weight females.

[2]A total of 2.27 gm. per chick was given orally over the four week period.

[3]Same as 2 except 4.54 gm.

Three males and three females were selected randomly from one replicate lot of each of five treatments to be used for the carcass composition studies.

Results

The results of Experiment XVI are shown in Tables 13, 14, 15 and 16. Oral supplements of methyl linoleate reduced four week average chick weight at either the 2.27 or 4.54 gm. per chick level as shown in Table 13. Methyl linolenate increased body weight with either level.

Table 14

Saponifiable and Nonsaponifiable Fractions of Cottonseed Oil, Liver Residue and Biopar C as Sources of Unidentified Growth Factors

Modification of Basal	Chick weight 4 weeks[1,2,3] Rep I	Rep II	Av.
Basal	120	147	133
Saponifiable fraction from 2.75% cottonseed oil	183	187	185
Nonsaponifiable fraction from 2.75% cottonseed oil	181	180	181
5.0% pork liver residue	199	183	191
3.0% Biopar C	165	162	164
5.0% pork liver residue + 3.0% Biopar C	230	227	229
52.0% ground yellow corn	249	233	241
100.0% I.S.C. broiler ration	221	225	223

Experiment XVI

[1]Twenty chicks started on each treatment.

[2]$\frac{\text{Average weight males plus average weight females}}{2}$

[3]Significant mean difference at 1% level as compared with basal equals 30 gm.

In Table 14, results of the chick growth studies with six sources of unidentified growth factors are presented. The two replicates of each treatment had average weights which were similar in most cases. All individual supplements and combinations produced highly significant improvements in the basal diet. The saponifiable and nonsaponifiable fractions of cottonseed oil were essentially equal as growth-promoters. There was relatively small response from feeding non-fat Biopar C, but pork liver residue produced chicks which were 30 per cent heavier than chicks fed the basal. It appears that the Biopar C and the pork liver residue possess separate growth factors, since a combination of the two supplements resulted in an increase over the basal that was almost equal to the sum of their separate responses. Yellow corn was superior to all supplements as a source of unidentified factors.

Feed efficiency was improved by all the treatments as shown in Table 15. The combination of pork liver residue and Biopar C, or the yellow corn produced maximum efficiency of feed utilization. The nonsaponifiable fraction of cottonseed oil improved feed efficiency more than the saponifiable fraction. The chicks receiving the basal diet consumed considerably less feed than those fed one or more of the various sources of unidentified factors in their diet. In general, the greater the weight of the chicks, the greater was the feed consumption.

Table 16 shows the results of the carcass composition studies. The chicks on the "fat-free" basal diet had a greater percentage of fat in their carcasses and a smaller

Table 15

Effect of Cottonseed Oil Fractions, Liver Fractions and Corn on Feed Consumption and Feed Efficiency

Modification of basal	Feed consumption[2] Replicates I	II	Av.	Feed efficiency Replicates[1] I	II	Av.
Basal	274	287	280	3.22	2.61	2.92
Saponifiable fraction from 2.75% cottonseed oil	406	398	402	2.78	2.64	2.71
Nonsaponifiable fraction from 2.75% cottonseed oil	372	336	354	2.58	2.35	2.46
5.0% pork liver residue	392	348	370	2.44	2.38	2.41
3.0% Biopar C	342	314	328	2.76	2.44	2.60
5.0% pork liver residue + 3.0% Biopar C	413	439	426	2.24	2.17	2.20
52.0% ground yellow corn	463	434	448	2.21	2.20	2.20

Experiment XVI

[1]Significant mean difference at the 5% level equals 0.47 and at the 1% equals 0.70 when compared with basal. Grams of feed per gram of gain.

[2]Grams feed per chick to four weeks of age.

Table 16

Unidentified Factors Increase Carcass Protein and Reduce Carcass Fat

Modification of basal	Per cent ether[1]-alcohol extract of moisture-free carcasses	Per cent protein[1,2] of moisture-free carcasses
Basal	51.04	34.51
5.0% pork liver residue + 3.0% Biopar C	35.94	42.22
Nonsaponifiable fraction from 2.75% cottonseed oil	32.81	48.24
52.0% ground yellow corn	24.82	48.31
Saponifiable fraction from 2.75% cottonseed oil	22.16	49.51

Experiment XVI

[1]Composite of three males and three females per group.

[2]Four-week old chicks

percentage of protein when measured on a moisture-free basis.

Experiment XVII

Objective

The objectives of this experiment were to determine whether (1) the ether-alcohol extract of ground yellow corn, the ether-alcohol extract of pork liver residue or the non-saponifiable fraction of cottonseed oil contained unidentified factors; (2) the oleic acid concentrate had a separate and distinct growth factor not found in corn or pork liver residue; (3) sources of unidentified factors affected body composition; (4) sources of unidentified factors affected weight of testes.

Methods

Two lots of ten male New Hampshire chicks were started on each experimental diet.

Results

As shown in Table 17, the ether-alcohol extract which was obtained by the hot extraction of pork liver residue was toxic to the chicks and reduced growth. On the other hand, the ether-alcohol extract of yellow corn had no effect on body weight. Only a slight growth response resulted from the inclusion of the nonsaponifiable fraction from 2.75 per cent cottonseed oil while the saponifiable fraction of the same amount of oil increased growth an average of 16 per cent. The chicks receiving the combination of pork liver residue and the oleic acid concentrate were 16 gm. heavier than those receiving only the pork liver residue as a supplement. However, supplementation with oleic acid concentrate alone was extremely toxic to the chicks and growth-depressing. The

Table 17

Cottonseed Oil Fractions, Pork Liver Residue, Oleic Acid Concentrate and Corn as Sources of Unidentified Growth Factors

Modification of basal	Per cent mortality at 4 wks.	Four wk. wts.[1] Rep 1	Rep 2	Av.
Basal	25	187	224	206
5.0% pork liver residue	10	222	213	218
1.0% oleic acid conc.	65	126	204	165
5.0% pork liver residue + 1.0% oleic acid conc.	0	224	243	234
Ether-alcohol (hot) extract from 5% pork liver residue	30	184	171	178
Saponifiable fraction from 2.75% cottonseed oil	15	274	214	244
Nonsaponifiable fraction from 2.75% cottonseed oil	15	212	219	216
25.0% ground yellow corn	0	304	290	297
25.0% ground yellow corn + 1.0% oleic acid conc.	5	244	313	278
Ether-alcohol (hot) extract from 25.0% ground yellow corn	40	204	208	206

Experiment XVII

[1]Significant mean difference at 5% level when any treatment is compared with basal equals 66 gm.

addition of the oleic acid concentrate to the diet containing 25 per cent yellow corn, also, appeared to slightly depress growth.

The average body weight at four weeks for the groups receiving 25 per cent ground yellow corn was the greatest that was obtained in all the experiments that are reported in these studies.

Either 1 per cent oleic acid concentrate or the alcohol-ether extract of the pork liver residue caused considerable mortality. The toxicity of the oleic acid concentrate was completely eliminated by adding pork liver residue to the diet.

Feed efficiency was approximately the same for all treatments except yellow corn which decidedly improved the feed efficiency as shown in Table 18. The chicks on the basal diet consumed approximately 8 and 9 per cent less feed, respectively, than those receiving the nonsaponifiable fraction of cottonseed oil or the pork liver residue. The addition of corn or the saponifiable fraction of cottonseed oil to the basal diet resulted in an increase in consumption of about 23 per cent.

The results of the carcass composition studies are recorded in Table 19. The chicks receiving the basal diet had the greatest amount of carcass fat. The chicks receiving the nonsaponifiable fraction of cottonseed oil which

Table 18

Effect of Cottonseed Oil Fractions, Liver Residue, Oleic Acid Concentrate and Corn on Feed Consumption and Efficiency

Modification of basal	Feed consumption[1] Replicates			Feed efficiency[2] Replicates		
	I	II	Av.	I	II	Av.
Basal	350	425	388	2.33	2.27	2.30
5.0% pork liver residue + 1.0% oleic acid conc.	454	440	447	2.43	2.13	2.28
5.0% pork liver residue	423	427	425	2.28	2.43	2.36
Saponifiable fraction from 2.75% cottonseed oil	568	458	513	2.39	2.58	2.48
Nonsaponifiable fraction from 2.75% cottonseed oil	431	405	418	2.47	2.23	2.35
25% ground yellow corn	514	499	506	1.93	1.97	1.95
25% ground yellow corn + 1.0% oleic acid conc.	442	499	470	2.13	1.81	1.97

Experiment XVII

[1]Grams feed per chick to four weeks of age.

[2]Grams feed per gram of gain to four weeks of age.

Table 19

Unidentified Factors Increase Carcass Protein and Reduce Carcass Fat

Modification of basal	Per cent ether[1] alcohol extract of moisture-free carcasses	Per cent protein[1,2] of moisture-free carcasses
Basal	29.76	53.08
5.0% pork liver residue + 1.0% oleic acid conc.	23.24	56.65
25% ground yellow corn	21.74	58.13
Saponifiable fraction from 2.75% cottonseed oil	22.53	57.45
Nonsaponifiable fraction from 2.75% cottonseed oil	26.64	54.44

Experiment XVII

[1]Determined on a composite sample of six males.

[2]Four-week old chicks.

contained no fatty acids had only 3 per cent less fat than the basal chicks. Supplements of pork liver residue plus oleic acid concentrate, yellow corn or the saponifiable fraction of cottonseed oil decreased the alcohol-ether extract from 6.5 to 8 per cent below the level obtained with the basal. The supplements to the basal diet had the opposite effect on carcass protein. Carcass protein was increased only 1.4 per cent by the nonsaponifiable fraction of cottonseed oil, while pork liver residue plus oleic acid concentrate, the saponifiable fraction of cottonseed oil and ground yellow corn raised the moisture-free carcass protein 3.5, 4.3 and 5.0 per cent, respectively.

The effects of several sources of unidentified factors on the weight of the testes of chicks are shown in Table 20. The chicks receiving the unidentified factors in their diets had larger testes. These increases in weight were in all cases matched by corresponding increases in body weight.

Experiment XVIII

Objective

Experiment XVIII had the following objectives: to establish the level of corn oil (Mazola) that would provide the required amounts of unidentified factors and to determine whether there was an interaction between procaine

Table 20

Effect of Unidentified Factors on Weight of Testes

Modification of basal	Per cent increase in body wt. over basal	Per cent increase[1,2] in wt. of testes over basal
Basal	0.0	0.0
Nonsaponifiable fraction from 2.75% cottonseed oil	20.5	15.0
5% pork liver residue + 1% oleic acid conc.	25.5	26.5
Saponifiable fractions from 2.75% cottonseed oil	37.9	38.4
25% ground yellow corn	57.2	50.6

Experiment XVII

[1]Six chicks, 4 weeks of age.

[2]Moisture-free testes.

penicillin G and the unidentified factors in corn oil.

Methods

Five male and five female New Hampshire chicks were started in each lot and the six treatments which were replicated are shown in Table 21. The analysis of variance for this experiment, which is recorded in the Appendix, is only for the replicated lots.

Table 21

Penicillin Promotes the Utilization of
Higher Levels of Corn Oil

Modification of basal	Two-to-four week gain (gm) With 5 p.p.m. penicillin		Without penicillin	
Basal	117	(191)[1]	126[2]	(209)
0.5% corn oil (Mazola)	97	(164)	121	(208)
1.0% corn oil	137	(206)	138	(209)
2.0% corn oil	148	(230)	133	(210)
4.0% corn oil	152	(237)	142	(225)
8.0% corn oil	161	(251)	138	(220)
3.0% Biopar C	138	(232)		
3.0% Biopar C + 4.0% corn oil	175	(269)		

Experiment XVIII

[1]Figures in parentheses represent four week weights in grams.

[2]Figures which are underlined are averages of two replicate lots.

[3]Significant mean difference of four week weights for replicated lots when compared with the basal is 41 gm. at the 5% level and 64 gm. at the 1% level.

Results

Procaine penicillin G when added to the "fat-free" basal or to 0.5 per cent corn oil diet appeared to depress growth as shown in Table 21. However, when corn oil was present at the 2, 4, or 8 per cent levels, penicillin additions had a growth-promoting effect. Without penicillin in the diet, 1 per cent corn oil was almost as satisfactory as higher levels, but in the presence of penicillin, the best growth was obtained with 8 per cent corn oil.

Three per cent Biopar C and 4 per cent corn oil increased the average two-to-four week gain by 21 and 35 gm., respectively, over the basal supplemented with penicillin. The growth-promoting effects appeared to be additive since a combination of Biopar C and 4 per cent corn oil improved the gain by 52 gm.

Table 22 contains the average weight of the testes. Four per cent corn oil had no effect on either testis weight or body weight of these chicks.

Table 22

Effect of Unidentified Factors on Weight of Testes

Modification of basal	No. chicks	Chick wt. 31 days	Av. wt. testes (mg)[1]
Basal	5	257	75
4% corn oil	8	264	72

Experiment XVIII

[1]Wet weight of pair of testes.

Experiment XIX

Objective

The objectives of this experiment were to determine: (1) whether the growth-promoting activity of the oleic acid concentrate was enhanced by refrigeration of the diet or inclusion of additional antioxidant in the diet; (2) whether hydrogenated soybean oil (oleomargarine) contains the unidentified factors present in soybean oil; (3) the effect on growth of animal and vegetable fats differing in their degree of unsaturation.

Methods

Two lots of five male and five female New Hampshire chicks were started on each diet. All feed was refrigerated at 44° F., except for a small amount always present in each feeder. The basal diet was modified to contain 5 p.p.m. procaine penicillin G.

Results

As shown in Table 23, 1 per cent oleic acid concentrate, 4 per cent soybean oil or a combination of two supplements produced highly significant increases in growth over the basal diet. One per cent oleic acid concentrate was not

Table 23

The Effect on Growth of Seven Animal or Vegetable Fats with Various Degrees of Unsaturation

Modification of basal	Average wt. 4 weeks		
	Rep 1	Rep 2	Av.[1]
Basal	191	199	195
1.0% oleic acid conc.[2]	239	272	256
100 mg./lb. alpha tocopherol acetate	200	231	216
1.0% oleic acid conc.[3] + 100 mg./lb. alpha tocopherol acetate	214	209	212
1.0% oleic acid conc. + 4.0% soybean oil	248	262	255
4.0% soybean oil	239	252	245
Liver residue ether extract (cold) from 5.0% pork liver residue	245	214	230
5.0% butter	228	225	226
5.0% oleomargarine	216	227	221
5.0% lard	---[4]	111	111

Experiment XIX

[1]Significant mean difference when any treatment is compared with the basal is 33 gm. and 47 gm. for the 5% and 1% levels, respectively.

[2]Animal fat obtained from beef tallow.

[3]An older sample of oleic acid conc. which apparently had deteriorated somewhat.

[4]No survivors at 4 weeks.

improved by the addition of 4 per cent soybean oil. Growth was improved by 13.8 and 11.8 per cent, respectively, by butter and oleomargarine. Lard caused high mortality by four weeks, but did produce small increases in body weight during the first two weeks. The more highly unsaturated oils (oleic acid concentrate, soybean oil and the cold extract of liver residue) were superior to butter and oleomargarine as growth-promoters.

Table 24 shows the feed efficiency and consumption data for this experiment. Feed efficiency was markedly improved by oleic acid concentrate, soybean oil or a combination of both. The liver residue extract also improved feed efficiency, but to a smaller extent. Oleomargarine did not improve feed efficiency. Butter improved it slightly, but was inferior to the more unsaturated oils in this respect.

Feed consumption was lower for the basal, liver residue extract and 1.0 per cent oleic acid concentrate plus 100 mg. of alpha tocopherol acetate per pound groups. The oleomargarine and 1 per cent oleic acid concentrate groups consumed the greatest amounts of feed.

Table 24

The Effect on Feed Efficiency and Consumption of Seven Animal or Vegetable Fats with Various Degrees of Unsaturation

Modification of basal	Feed consumption[1] Replicates			Feed efficiency[2,3] Replicates		
	I	II	Av.	I	II	Av.
Basal	373	363	368	2.42	2.24	2.33
1.0% oleic acid conc.	383	470	426	1.90	2.00	1.95
100 mg./lb. alpha tocopherol acetate	427	373	400	2.20	2.28	2.24
1.0% oleic acid conc. + 100 mg./lb. alpha tocopherol	386	387	386	2.24	2.19	2.22
1.0% oleic acid conc. + 4.0% soybean oil	422	408	416	2.00	1.82	1.91
4.0% soybean oil	380	418	399	1.88	1.94	1.91
Liver residue ether extract (cold) from 5.0% pork liver residue	373	394	384	1.80	2.22	2.01
5.0% butter	404	409	406	2.11	2.18	2.14
5.0% oleomargarine	413	431	422	2.31	2.27	2.29

Experiment XIX

[1]Grams of feed per chick to four weeks of age.

[2]Grams feed per gram of gain to four weeks of age.

[3]Significant mean difference when compared with basal is 0.29, 0.37 and 0.42 for the 5%, 2% and 1% levels, respectively.

[4]No data are shown for lard because of the high mortality occurring in lots receiving this supplement.

DISCUSSION

Unidentified Growth Factors in Vegetable Oils

Chick weight at four weeks of age was increased from 10 to 30 per cent by the addition of refined and crude corn oils, soybean oil, wheat germ oil or cottonseed oil to a semi-purified "fat-free" diet in these studies. The failure of Russell, Taylor and Polskin (1940) to depress growth by extraction of the fat from a chick-starter diet was possibly due to the presence of fatty materials which were not removed by their method of extraction. It is known that it is extremely difficult to remove all the fat from a diet composed of natural feedstuffs.

In the present studies a noticeable growth response to the vegetable oils did not occur until the chicks were about two weeks of age. The absence of a significant response during the initial two-week period is explainable if a carry-over of the unidentified factors from the dam through the egg to the chick is postulated. It is conceivable that the breeder diets used by Russell, Taylor and Polskin (1940), and Davis and Upp (1941), were of a higher fat content or more potent in factors associated with fats and thus caused considerable storage of the unidentified factors in the hens' progeny.

Refined and crude corn oils, soybean oil, wheat germ oil and cottonseed oil have been shown to be good sources of unidentified growth factors in these experiments. Reiser (1950a) found that cottonseed oil improved growth of chicks fed a semi-purified diet and that 4 per cent was superior to 1 per cent. He stated that the growth response to the cottonseed oil was due to its content of polyunsaturated fatty acids although no direct proof of this statement was presented.

The saponifiable fraction of cottonseed oil (Experiments XVI and XVII) contained the major portion of the growth-promoting activity found in cottonseed oil, but smaller responses were obtained in both experiments with the non-saponifiable fraction of the oil. Therefore, it appears that the fatty acids or unknown vitamins present in fats are responsible for most of the growth-promoting action of fats in the present study.

The growth factors found in vegetable oils are not required to prevent dermatitis since this condition was not found in chicks fed the "fat-free" diet. Livability was excellent on the basal "fat-free" diet except in those experiments in which all pens suffered abnormal mortality.

This is contrary to the findings of Reiser (1950a). It should be pointed out, however, that mortality was high even in Reiser's fat-supplemented pens.

Wheat germ oil was equally effective in stimulating growth when present in the mixed feed or when orally fed by syringe (Experiment XV). The growth response to orally fed wheat germ oil suggests that vegetable oils do not increase growth by improving the palatability of the feed, since the chicks ate sufficient feed to increase growth even though wheat germ oil was not mixed in the basal diet. The failure of orally administered oleic acid concentrate to improve growth was possibly due to the fact that it is composed of free fatty acids. According to Quackenbush, Steenbock, Kummerow and Platz (1942), free fatty acids are exceedingly irritating to the digestive tract of the rat. It was observed in the present studies that inflammation of the skin around the mouth occurred in some of the chicks receiving the oleic acid concentrate.

Commercially hydrogenated soybean oil in the form of oleomargarine is devoid of much of the unidentified growth factor activity possessed by soybean oil indicating that unsaturated fatty acids or other unsaturated compounds in the soybean oil are its principal growth-promoting factors (Experiment XIX). Commercial hydrogenation of soybean oil is not complete, leaving approximately 2 to 5 per cent "essential" polyunsaturated fatty acids intact according to Woods (1948). This may explain the small growth response to the oleomargarine or it may be due to the same factor(s) that are present in the nonsaponifiable fraction of cottonseed oil as demonstrated in Experiments XVI and XVII. The oleomargarine increased consumption of the diet, but feed efficiency was the same as the basal.

The requirement for the growth factors present in vegetable oils is satisfied with the addition of from 1 to 2 per cent corn oil, soybean oil, cottonseed oil or wheat germ oil. These vegetable oils appear to contain similar quantities of the same growth factors and thus, the addition of 2 per cent of both corn oil and soybean oil gave no greater growth response than either alone (Experiment IX). In Experiments XVIII and XIX penicillin appeared to increase the requirement for the unidentified factors which were deficient in the basal diet. As shown in Table 21, penicillin increased growth when corn oil was supplied at the 2 per cent level. These results suggest that the growth response to penicillin occurs only when a certain quantity of fat is in the diet or only with an adequate diet, but definite conclusions can not be drawn from these limited data.

Unidentified Growth Factors in Fatty Acid Concentrates

Sixty per cent linoleic acid concentrate

The growth response to the linoleic acid concentrate was inconsistent in these studies. This was apparently due to its tendency to become rancid as shown by the Kreis rancidity test. In Experiments VIII and X the linoleic acid concentrate definitely was growth-promoting and improved growth 23 and 16 per cent. One and a half per cent linoleic acid concentrate was superior to a level of 0.025 or to a combination of 0.5 per cent linoleic acid concentrate plus 0.025 alpha tocopherol. Low levels of the concentrate are adequate when they are fed in combination with low levels of wheat germ oil (Experiments VIII and XII). It appears that wheat germ oil and linoleic acid concentrate contain two distinct growth factors with each oil possessing both factors, but with a different one of the two factors existing in greater concentration in each oil. This accounts for the maximum response obtained with a combination of low levels of the two oils. If only one oil was fed, a higher level was needed to provide a sufficient amount of the marginal factor in that particular oil.

The linoleic acid concentrate was consistently toxic when used as a supplement to the vitamin-test casein basal, and it never produced an increase in growth. The industrial soybean protein apparently contains antioxidant substances which under certain conditions prevent the breakdown of the fatty acids to form toxic peroxides, aldehydes and ketones. Experiment X is an excellent example of the converse effects of the concentrate in the vitamin-test casein and industrial soybean protein basal diets. The concentrate improved growth and caused no mortality as a supplement to the soybean protein basal but retarded growth and caused high mortality when added to the casein basal. However, it should be emphasized that this concentrate is toxic in either basal diet unless a freshly prepared sample of the oil is used. The oil deteriorates even under refrigerated conditions.

Oleic acid concentrate

The oleic acid concentrate was capable of eliciting a maximum growth response in these experiments. Since 1 per cent of the oleic acid concentrate supplies the basal diet with less than 0.05 per cent linoleic acid, it appears that linoleic acid is not the growth-promoting factor in this concentrate. Oleic acid could possibly be one of the growth factors in this concentrate but no direct proof is available to support this hypothesis. Furthermore, there appeared to be no correlation between the oleic acid content of vegetable oils and their growth-promoting action. One per cent was consistently the optimum level for feeding the concentrate throughout these experiments.

No improvement in growth occurs when a vegetable oil is added to a diet which contains 1 per cent oleic acid concentrate (Experiment XIX), unless the concentrate is approaching an early stage of rancidity such as in Experiment XV. In such cases, the antioxidant properties of vegetable oils prevent any breakdown of the fatty acids into toxic products and allow the concentrate to function normally. Also, in Experiments XIV and XVII, where negative results were obtained when the concentrate was fed as the only supplement to the basal, growth responses resulted with a combination of 1 per cent oleic acid concentrate and 25 per cent corn, or the concentrate plus 5 per cent pork liver residue. The corn and the pork liver residue apparently contained antioxidants which prevented the deterioration of the concentrate.

Pure Fatty Acids as Chick Growth Stimulators

Since vegetable oils and the fatty acid concentrates used in these studies are comprised almost entirely of fatty acids, the question arises whether specific fatty acids are responsible for the growth-promoting properties of vegetable oils and the fatty acid concentrates.

Stearic acid, which is found in varying concentrations in vegetable oils, was shown to have no effect on chick growth (Experiment XIII). The failure of stearic acid, a saturated fatty acid, to improve growth indicates that the fat requirements of the chick are specific and probably limited to unsaturated fatty acids. The relatively small growth responses produced by oleomargarine and butter (Experiment XIX), which contain only small amounts of unsaturated fatty acids, supports the hypothesis that the growth-promoting properties of fats are concentrated in the unsaturated fatty acids.

Pure methyl linoleate increased growth at either of two levels in Experiment XIII. Methyl oleate had little effect on growth rate, but only a total of six chicks were involved in the experiment. In Experiment XVI, larger numbers of chicks were used. Unfortunately, growth was below normal and mortality was slightly higher in all lots, including the positive control. Therefore, the results of the pure fatty acid studies may have been partially confounded by other factors. Contrary to the findings of Experiment XIII, methyl linoleate retarded growth while methyl linolenate increased the growth of chicks. From these limited data, it appears that the response of chicks to methyl linoleate, methyl linolenate and methyl oleate is rather inconsistent and therefore it is not justifiable to state whether any one of these fatty acids specifically are required, or whether they are replaceable by other unsaturated fatty acids.

Other Sources of Unidentified Growth Factors

Liver L and Biopar C, which are both fat-free concentrates of liver, contain a growth factor(s) which is not present in either the basal diet or in fats (Experiments XII and XVIII). This factor(s) consistently increased chick growth, but only to a small extent.

Arachidonic acid was 3.5 times more effective than linoleic acid for stimulating growth of the rat, according to Greenberg, Calbert, Deuel and Brown (1951). Since approximately 18.8 per cent of the total fatty acids of pork liver residue were found to be arachidonic acid, the growth-promoting activity of pork liver residue was studied. The response to the liver residue was relatively small (Experiments XVI and XVII) when compared with ground yellow corn. Also, the addition of 1 per cent oleic acid concentrate to a diet containing 5 per cent pork liver residue further improved growth. This indicates either that the oleic acid concentrate contains an additional factor or that liver residue contains relatively small quantities of the growth factors. A hot extraction of the pork liver residue with a 3:1 alcohol-ether mixture either destroyed the growth factors of the residue or produced toxic substances (Experiment XVII). In Experiment XIX, however, a 15 per cent improvement in growth was obtained with a cold ether extract from 5 per cent pork liver residue. These results demonstrate that the growth factors in pork liver residue are ether-soluble.

In Experiment XIV, 25 per cent yellow corn appeared to provide insufficient quantities of the growth factors, since growth and feed efficiency were improved by the further addition of 1.0 per cent oleic acid concentrate. However, supplements of 25 per cent corn in Experiment XVII and 52 per cent corn in Experiment XVI produced maximum growth and feed efficiency. Therefore, it is concluded that corn is a good source of the unidentified factors. Evidence was not obtained from these experiments to establish whether a growth factor, other than those found in the fat, is present in corn.

Butter and oleomargarine were approximately equal as fat supplements for chicks (Experiment XIX). These results are similar to the findings of Lassen and Bacon (1949), who found that there was no difference between butter and oleomargarine as fat supplements for rats. Neither of these relatively hard fats is a good source of unidentified factors for the chick when compared with soybean oil or oleic acid concentrate.

Effect of Unidentified Factors on Carcass Composition and Weight of Testes

Carcass protein is increased and carcass fat is reduced by the addition of unidentified growth factors to the "fat-free" basal diet (Experiments XVI, XVII). In Experiment XVI, the differences between the basal group and others were quite large, but it should be pointed out that the growth of the basal chicks was extremely poor. Experiment XVII probably reflects the true situation more accurately. The differences between the basal and supplemented groups is still present but less pronounced -- especially in regard to the fat content of the carcasses. An example of the difference is the moisture-free carcass fat and protein of the chicks receiving the saponifiable fraction from 2.75 per cent cottonseed oil. Their carcasses contained 7.23 per cent less fat and 4.37 per cent more protein than the basal chicks. In Experiment XVII the rise in carcass protein and reduction in carcass fat appeared to be highly correlated with the increase in body weight. The group with the greatest body weight had the highest per cent of carcass protein and the least amount of carcass fat. These data suggest the hypothesis that the growth factors in fat do not function by increasing the formation of body fat but improve growth by accelerating general tissue metabolism.

Hormonal activity was not demonstrated for the various sources of unidentified factors in Experiment XVII. Weight of the testes appeared to be dependent on body weight with similar differences both in testes and body weight between the basal and any supplemented group.

SUMMARY

Sources of unidentified growth factors for chicks have been studied.

Crude or refined corn oil, wheat germ oil, soybean oil and cottonseed oil were sources of unidentified growth factors which increased chick weight at four weeks from 10 to 30 per cent. Factors in fat were not required for livability of chicks or for the prevention of dermatitis. The requirement of the chick for the growth factors in these vegetable oils was satisfied by the addition of from 1 to 2 per cent of any one of these oils to the basal diet. The four vegetable oils contained similar quantities of the unidentified factors. The saponifiable fraction of cottonseed oil possessed the major portion of its growth factors, but the nonsaponifiable fraction was also growth-promoting. Commercially hydrogenated soybean oil in the form oleomargarine was devoid of much of the growth factor activity possessed by soybean oil.

Growth responses to the 60 per cent linoleic acid concentrate were inconsistent. This may have been due to its decided tendency to become rancid. One and a half per cent of this concentrate in the diet was superior to lower levels

for increasing growth. Lower levels of the concentrate were adequate when they were fed in combination with low levels of wheat germ oil.

An oleic acid concentrate prepared from beef tallow was consistently slightly superior as a source of growth factors to other individual supplements of vegetable oils or the linoleic acid concentrate. The optimum level of feeding the oleic acid concentrate was 1 per cent. The addition of a vegetable oil to a diet containing 1 per cent of the oleic acid concentrate did not stimulate growth.

Growth response from feeding pure methyl oleate, methyl linoleate and methyl linolenate was inconsistent and rather small. Stearic acid was not growth-promoting for the chick at the 1 or 2 per cent levels.

The unidentified factors in fats and fatty acid concentrates were distinct from the growth factors shown by other workers to be present in fat-free Liver L and Biopar C. The factors in Liver L and Biopar C increased growth only slightly.

Pork liver residue, which contains 18.8 per cent arachidonic acid of its total fatty acids, was a fair source of growth factors which were shown to be ether-soluble.

Yellow corn increased growth and feed efficiency but this study did not establish whether this was entirely attributable to factors in the ether-soluble portion of the corn.

Butter and oleomargarine were approximately equal as fat supplements for chicks, but were inferior to more unsaturated fats.

Carcass protein was increased and carcass fat was reduced by the addition of unidentified growth factors to the "fat-free" diet. No hormonal activity, as reflected by weight of testes, could be demonstrated for the various sources of unidentified growth factors.

CONCLUSIONS

1. The chick requires unidentified growth factors present in fats or fatty acid concentrates for maximum growth and feed efficiency to four weeks of age.

2. The unidentified factors in fats or fatty acid concentrates are not required for livability of the chick or prevention of dermatitis.

3. Crude or refined corn oil, wheat germ oil, soybean oil and cottonseed oil are sources of chick growth factors with an optimum level in the diet of from 1 to 2 per cent.

4. The greater portion of the growth factors in cottonseed oil is in the saponifiable fraction.

5. An oleic acid concentrate is slightly superior to other oils as a source of growth factors and 1 per cent is its optimum level in the diet.

6. The unidentified factors in fats and fatty acid concentrates increase carcass protein and reduce carcass fat.

7. The chick does not have a specific requirement for linoleic or linolenic acids for growth.

8. Butter and oleomargarine are of approximately equal value as fat supplements, but are inferior to more unsaturated fats.

9. The unidentified factors in fats and fatty acid concentrates are distinct from the growth factors in Liver L and Biopar C.

10. Stearic acid does not have chick growth-promoting properties.

11. Pork liver residue contains ether-soluble growth factors.

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APPENDIX

A. Mineral Mixes

1. Mineral Mix A

Compound	gm.
$Ca\ H\ PO_4 \cdot 2\ H_2O$	2,516.2
$K_2\ H\ PO_4$	840.0
$Ca\ CO_3$	700.0
$Na\ Cl$	600.0
$MgSO_4$	500.0
$Mn\ SO_4$ Mix (65% $Mn\ SO_4$)	29.0
Zn Acetate	29.0
Cobalt Acetate	0.2
Iron Sulfate	55.0
K I	3.5
$CuSO_4 \cdot 5\ H_2O$	1.5
TOTAL	5,274.4

This was added at the 5.0% level to the total diet.

2. Mineral Mix B

Compound	lb.	gm.
$Ca\ H\ PO_4 \cdot 2\ H_2O$	12.20	
$K_2\ H\ PO_4 \cdot 3\ H_2O$	2.85	
$Ca\ CO_3$	3.08	
$Na\ Cl$	2.20	
$Mg\ SO_4$	1.05	
Zn Acetate		58
Cobalt Acetate		2
Iron Sulfate		110
$Mn\ SO_4$ Mix (65% $Mn\ SO_4$)		58
K I		7
$CuSO_4 \cdot 5\ H_2O$		3

This was added at the 5.5% level to the total diet.

B. Analyses of Variance

1. Experiment IX
   Three-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	130	130
Treatments	9	1,373	153
Interaction	9	871	97
TOTAL	19	2,374	

2. Experiment XII
   Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Treatments	7	4,176	597
Between pens treated alike	8	2,575	322
TOTAL	15	6,751	

3. Experiment XIII
   Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Treatments	5	4,406	881
Between pens treated alike	6	1,737	289
TOTAL	11	6,143	

4. Experiment XIV
   Three-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Treatments	4	1,407	352
Between pens treated alike	5	464	93
TOTAL	9	1,871	

5. Experiment XV
Two-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	11	
Treatments	7	1,783	255*
Interaction	7	346	49
TOTAL	15		

6. Experiment XVI
a. Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Treatments	7	18,197	2,600**
Between pens treated alike	8	646	81
TOTAL	15		

b. Feed efficiency of chicks

Source of Variation	d.f.	S.S.	M.S.
Treatments	6	0.8194	0.1366
Between pens treated alike	7	0.2778	0.0397
TOTAL	13		

7. Experiment XVII
Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	696	696
Treatments	9	30,427	3,381*
Interaction	9	7,647	850
TOTAL	19	38,770	

*Significant at .05 level of probability
**Significant at .01 level of probability

8. Experiment XVIII
Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	19	19
Treatments	5	6,906	1,381*
Interaction	5	1,260	252
TOTAL	11	8,184	

9. Experiment XIX
a. Four-week weight of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	201	201
Treatments	8	6,709	839*
Interaction	8	1,597	200
TOTAL	17	8,507	

b. Feed efficiency of chicks

Source of Variation	d.f.	S.S.	M.S.
Reps.	1	0.00436	0.00436
Treatments	8	0.45110	0.05639*
Interaction	8	0.13072	0.01634
TOTAL	17	0.58618	

*Significant at .05 level of probability